Oxidative stress measured by thioredoxin reductase level as potential biomarker for prostate cancer

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Abstract: The aims of this study were to determine if Thioredoxin reductase (TR) is detected in the serum, and to establish the sensitivity and specificity of serum TR for diagnosing prostate cancer (PC). We assessed serum TR in 380 participants in the training cohort: 160 patients with PC, 120 with benign prostatic hyperplasia and 100 healthy individuals. The validation cohort comprised 320 participants: 120 with PC, 100 with BPH and 100 healthy individuals. TR was measured in serum by ELISA by independent researchers. The patients with PC were graded using the Gleason system. Receiver operating characteristic (ROC) curves were utilized to evaluate the accuracy of biomarkers to diagnose PC. The influence of serum levels of TR on tumor grade and metastasis was performed by binary logistic regression analysis. The median levels of serum TR in PC were significantly higher than that of healthy subjects and patients with BPH (P < 0.0001). Based on the ROC curve, the optimal cutoff value of serum TR levels as an indicator for auxiliary diagnosis of PC from BPH was projected to be 8.2 U/ml, which yielded a sensitivity of 81.8% and a specificity of 68.9%, with the area under the curve at 0.862 (95% CI, 0.821-0.903). Combined model (TR and PSA) showed a significantly greater discriminatory ability as compared with those markers alone. In regression analysis, after adjusting for other significant predictors, TR remained an independent metastasis predictor with an adjusted OR of 4.99 (95% CI, 2.64-8.09). Similarly, TR also was an independent High-grade tumors (HGT) predictor with an adjusted OR of 5.15 (95% CI, 2.52-9.14). Our study has demonstrated the additional benefit of TR measurement in the diagnosis of PC in the Chinese population. Further studies of the application of TR in this region may be beneficial.

Keywords: Thioredoxin reductase, prostate cancer, biomarker, diagnosis, Chinese

Introduction

Worldwide, prostate cancer (PC) is the second-most frequently diagnosed cancer [1]. The American Cancer Society estimates that in 2011, 240,890 men were diagnosed with prostate cancer and 33,720 men died of it [2]. In Asian countries, the reported incidence rate and its subsequent mortality have been on the rise [3]. This pattern was mainly driven by Eastern Asia, and in particular China, which represents about 62% of the region's male population [4]. In China, although the incidence of PC is much lower than that in the U.S., the incidence is increasing rapidly, especially in metropolitan areas [5].

The incidence rates and 5-year survival rates are heavily influenced by the introduction of serum prostate-specific antigen (PSA) and widespread screening [2]. Although it remains controversial, screening appears to be effective in reducing mortality from PC, especially if screening and treatment are freely available to all patients [6]. The American Cancer Society recommends that beginning at age 50, a PSA blood test and digital rectal examination (DRE) should be offered to men annually [7]. Unfortunately, PSA screening cannot distinguish the early stages of PC from benign prostatic hyperplasia (BPH) effectively, especially when the PSA levels are within 4-10 ng/ml range. Biological evidence suggests that benign prostatic hyperplasia and prostatic carcinoma share common predisposing factors [8]. Although the possibility as biomarkers for PC has been investigated for some molecules, such as cirDNA epigenomics [9], cir miRs [10], PARP-1 and PAR [11], human glandular kallikrein 2 (hK2), urokinase plasminogen activator...
Table 1. Baseline characteristics of the PC and control cases

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Test cohort</th>
<th>Validation cohort</th>
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<tbody>
<tr>
<td></td>
<td>PC</td>
<td>BPH</td>
</tr>
<tr>
<td>N</td>
<td>160</td>
<td>120</td>
</tr>
<tr>
<td>Age (IQR, years)</td>
<td>68 (55-82)</td>
<td>68 (55-81)</td>
</tr>
<tr>
<td>Race, Han (%)</td>
<td>151 (94.4)</td>
<td>112 (93.3)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>102 (63.8)</td>
<td>75 (62.5)</td>
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<tr>
<td>Family history (%)</td>
<td>40 (25.0)</td>
<td>21 (17.5)</td>
</tr>
<tr>
<td>BMI (IQR, kg/m²)</td>
<td>25.5 (23.2-27.6)</td>
<td>25.7 (23.6-27.5)</td>
</tr>
<tr>
<td>DRE (+, %)</td>
<td>95 (59.4)</td>
<td>34 (28.3)</td>
</tr>
<tr>
<td>Metastasis (%)</td>
<td>45 (28.1)</td>
<td>-</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 7</td>
<td>55 (34.4)</td>
<td>-</td>
</tr>
<tr>
<td>7 (3+4)</td>
<td>42 (26.3)</td>
<td>-</td>
</tr>
<tr>
<td>7 (4+3)</td>
<td>33 (20.6)</td>
<td>-</td>
</tr>
<tr>
<td>&gt; 7</td>
<td>30 (18.7)</td>
<td>-</td>
</tr>
<tr>
<td>Lab findings (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum PSA (ng/ml)</td>
<td>8.2 (3.2-21.5)</td>
<td>6.3 (2.4-12.3)</td>
</tr>
<tr>
<td>0-4.0</td>
<td>49 (30.6)</td>
<td>45 (37.5)</td>
</tr>
<tr>
<td>4.1-20.0</td>
<td>70 (43.8)</td>
<td>66 (55.0)</td>
</tr>
<tr>
<td>&gt; 20.0</td>
<td>41 (25.6)</td>
<td>9 (7.5)</td>
</tr>
<tr>
<td>Hs-CRP (mg/dl)</td>
<td>0.44 (0.28-0.57)</td>
<td>0.35 (0.24-0.47)</td>
</tr>
</tbody>
</table>

PC = Prostate cancer; PSA = Prostate-specific antigen; DRE = Digital rectal examination; BPH = Benign prostatic hyperplasia; TR = Thioredoxin reductase; BMI = Body mass index; Hs-CRP = High-sensitivity-C-reactive protein; IQR = Interquartile ranges.

(uPA), transforming growth factor-beta 1 (TGF-β1), KLK2, PSMA and prostate cancer gene 3 (PCA3), their prospects in clinical application still need to be further evaluated [12-15]. The need for additional biomarkers that supplement PSA is reflected by the number of ongoing studies in this field.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are not only byproducts of normal cellular metabolism, but also play important roles in cell signaling. Studies have shown that oxidative stress (OS) conditions play an important role in both the initiation and the progression of prostate cancer by regulating molecules such as DNA, enhancers, transcription factors, and cell cycle regulators [16]. The relevance of OS in prostate carcinogenesis is suggested by associations between prostate cancer and conditions associated with OS as well as medications and nutrients that affect OS level [17]. Several studies have identified a link between OS and PC using tissue level markers such as 8-hydroxydeoxyguanosine [18, 19].

Redox imbalance has long been recognized to be a factor in the pathology of PC [20]. One of the major redox control systems is the thioredoxin system comprised of thioredoxin (TRX) and thioredoxin reductase (TR) [21]. The mammalian TR are a family of selenium-containing pyridine nucleotide-disulphide oxidoreductases with mechanistic and sequence identity, including a conserved-Cys-Val-Asn-Val-Gly-Cys-redox catalytic site, to glutathione reductases [22]. TRs catalyse the NADPH-dependent reduction of the redox protein TRX, as well as of other endogenous and exogenous compounds. Secretion of TR under conditions of oxidative stress and inflammation has been observed from many neoplastic cells [23]. TR overexpression has been reported in several malignancies (colorectal, prostate and hepatocellular carcinomas) and may be associated with aggressive tumor growth and poor outcome [24-26]. However, information regarding its utility in the Chinese is scanty. The aim of our study was to determine whether TR were detectable in serum, and whether the TR can distinguish PC from age-matched BPH or healthy controls. Finally, the diagnosis value of TR combined with routine biomarkers such as PSA and DER was also investigated.

Patients and methods

Patients

In our study, two cohorts of patients were enrolled. Training cohort recruited 160 consec-
Review Board of the Zhongnan Hospital of Wuhan University. Written informed consent was obtained from each inductee in accordance with the Ethics Committees Guidelines for our institution.

Clinical variables

Demographic and pre-biopsy clinical parameters, including age, race, history of smoking, height, weight, Body mass index (BMI), family history of PC, history of previous prostate biopsy, DRE findings, pre-biopsy PSA and High-sensitivity-C-reactive protein (Hs-CRP) were obtained using interviewer-administered questionnaires and abstracting data from patients' medical records. The patients with PC were graded using the Gleason system [28]. High-grade tumors (HGT) were defined as Gleason scores 8 to 10 and 7 (4 + 3); low-grade tumors (LGT) were Gleason scores 2 to 6 and 7 (3 + 4).

Laboratory testing

The preoperative serum PSA and TR levels were simultaneously measured in the patients using standard methods at admission. Venous blood samples were taken in the morning's fasting state. After at least 30 min, but within 2 h, the tubes were centrifuged at 20°C for 15 min at 1,200 g, and the sera were stored frozen in plastic vials at -80°C until the time of consecutive analyses. The controls samples were collected and stored in the same way as the PC samples. PSA levels were measured with commercially available immunoassay methods by DPC Immulite 2000 (Diagnostic Products Corporation, CA, USA). A cut-off value of 4 ng/mL was used. Serum levels of TR were measured in duplicate using a solid-phase sandwich ELISA that uses two highly specific antibodies to human TrxR protein (BioVision Incorporated, Milpitas Boulevard, Milpitas, CA, USA) according to the manufacturer's instruction. The coefficients of variation (CVs) of inter-assay and intra-assay were 4.8-8.0% and 6.2-9.0%, respectively. The lower detection limit was 0.5 U/ml and the line range was 0.5-100 U/ml. For all measurements, levels that were not detectable were considered to have a value

Figure 1. Serum levels of TR in patients with PC and controls. All data are medians and interquartile ranges (IQR). Mann-Whitney U-test. PC = Prostate cancer; BPH = Benign prostatic hyperplasia; TR = Thioredoxin reductase.

Patients with PC and BPH were initial diagnosis without any treatment. Subjects with other conditions, which may alter TR, such as previous/concomitant other neoplasms, inflammatory, diabetes, chronic kidney disease, severe burn injury and cardiovascular diseases, were excluded. The diagnosis for each case including BPH and PC was confirmed by fine needle aspiration biopsy (FNA) histopathology examination by the urologist using the National Comprehensive Cancer Network (NCCN) guidelines [27]. For bone metastasis, the PC patients would undergo further emission computed tomography (ECT) bone scan.

The healthy age-matched men from routine physical exam including DRE, B-mode ultrasound prostate scan (BMUP), and International Prostate Symptom Score (I-PSS) were selected as normal control population. There were no significant differences in demographic or clinicopathological characters of patients with PC between the training and validation cohorts. This study was approved by the Institutional
TR level as potential biomarker for PC

**Table 2.** Results for measurement of serum TR, PSA, or both* in the diagnosis of PC

<table>
<thead>
<tr>
<th></th>
<th>Training cohort</th>
<th>Validation cohort*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>AUC</td>
<td>95% CI</td>
</tr>
<tr>
<td>PC vs. BPH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>0.862</td>
<td>0.821-0.903</td>
</tr>
<tr>
<td>PSA</td>
<td>0.626</td>
<td>0.521-0.690</td>
</tr>
<tr>
<td>DRE</td>
<td>0.723</td>
<td>0.644-0.805</td>
</tr>
<tr>
<td>Combined (TR + PSA)</td>
<td>0.904</td>
<td>0.854-0.947</td>
</tr>
<tr>
<td>PC vs. BPH and normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>0.879</td>
<td>0.828-0.917</td>
</tr>
<tr>
<td>PSA</td>
<td>0.702</td>
<td>0.643-0.785</td>
</tr>
<tr>
<td>DRE</td>
<td>0.729</td>
<td>0.684-0.838</td>
</tr>
<tr>
<td>Combined (TR + PSA)</td>
<td>0.911</td>
<td>0.861-0.955</td>
</tr>
</tbody>
</table>

*In the validation cohort, the diagnostic cutoff values of serum TR and PSA were 8.2 U/mL and 4.0 ng/mL respectively. PC = Prostate cancer; PSA = Prostate-specific antigen; DRE = Digital rectal examination; BPH = Benign prostatic hyperplasia; TR = Thioredoxin reductase; OR = Odds ratios; CI = Confidence interval; ROC = Receiver operating characteristic; AUC = Area under the curve. *TR + PSA.

**Statistical analysis**

Results are expressed as percentages for categorical variables and as medians (interquartile ranges, IQRs) for the continuous variables. Proportions were compared using the χ² test, and the Mann-Whitney test to compare continuous variables between groups. Correlations were determined using Spearman critical value rankings. Receiver operating characteristic (ROC) curves were utilized to evaluate the accuracy of biomarkers to diagnose PC. Area under the curve (AUC) was calculated as measurements of the accuracy of the test. The influence of serum levels of TR on tumor grade and metastasis was performed by binary logistic regression analysis, which allows adjustment for other confounding factors, such as, age, family history, history of smoking, BMI, DRE findings and serum levels of PSA and Hs-CRP. Results were expressed as adjusted OR (odds ratios) with the corresponding 95% CI (Confidence interval). All statistical analysis was performed with SPSS for Windows, version 19.0 (SPSS Inc., Chicago, IL, USA) and the ROCR package (version 1.0-2), which is available from CRAN repository (http://cran.r-project.org/). Two-tailed significance values were used and significance levels were set at 0.05.

**Results**

In our study, we recruited 700 participants overall, 380 in the test cohort (160 with PC) and 320 in the validation cohort (120 with PC).
In the test cohort, the median ages of the PC were 68 (range, 55-82) years. The median serum PSA and TR levels of the normal group were 3.4 (IQR, 1.8-6.0) ng/ml and 5.1 (3.0-7.2) U/ml, respectively. In the 160 patients, 45 (28.1%) were with metastasis. Baseline characteristics of the PC and control cases were shown in Table 1.

**Main findings**

The median levels of serum TR was 16.1 (8.0-25.3) U/ml, which was significantly higher than that of healthy subjects and patients with BPH ($P < 0.0001$; Figure 1). Similarly, TR was also significantly higher as compared to controls in validation cohort (Table 1). Although the median level of PSA in serum was increased for patients in the PC group compared with that in healthy controls, as expected ($P < 0.0001$), significant increases were also seen in patients with BPH ($P < 0.0001$; Table 1). In addition, there was a weak but significant positive correlation between TR and Hs-CRP ($r = 0.215, P < 0.001$). Statistical analysis here revealed no influence of age, DRE and PSA on TR in PC patients ($P > 0.05$, respectively). However, there was a positive correlation between PSA and age ($r = 0.203, P < 0.001$). Similarly results were obtained in the validation cohort (data not list).

**TR has higher sensitivity and specificity than PSA in diagnosis of PC**

A ROC curve was plotted to define the optimal cut-off values, and to identify the sensitivity and specificity of serum TR and PSA levels in differentiating patients with PC versus other conditions. Based on the ROC curve, the optimal cutoff value of serum TR levels as an indicator for auxiliary diagnosis of PC from BPH was projected to be 8.2 U/ml, which yielded a sensitivity of 81.8% and a specificity of 68.9%, with the area under the curve at 0.862 (95% CI, 0.821-0.903; Table 2 and Figure 2). Thus, we chose 8.2 U/ml as the cutoff value for TR in this study. The optimum cutoff value for PSA was 3.8 ng/mL (AUC 0.626, 95% CI: 0.521-0.690, sensitivity 69.8%, specificity 42.0%). As the cutoff value was similar to those for the recommended clinical cutoff of 4.0 ng/mL, we chose 4.0 ng/mL as the cutoff value for PSA in this study. Predictive values for TR and PSA in the diagnosis of PC are shown in Table 2. TR had a better AUROC compared with PSA ($P < 0.001$), indicating both a higher sensitivity and specificity of TR compared with PSA in the diagnosis of PC (Figure 2). Combined model (TR and PSA) increased the sensitivity for PC to 72.5%, with a specificity of 95.2%, and the AUROC was 0.904 (95% CI: 0.854-0.947; Table 2). Combined
model showed a significantly greater discriminatory ability as compared with those markers alone (Table 2). Similarly, when PC patients were compared with BPH and normal cases, the AUC for TR was also larger than that for PSA (0.877, 0.818-0.920 vs. 0.702, 0.643-0.785, P < 0.001; Table 2).

The diagnostic value of TR in diagnosis of PC in the validation cohort

In the validation cohort, using the optimal cutoffs derived from the ROC curve, the positive predictive value of TR (> 8.2 U/ml) was 84.6%, while was 58.3% for PSA. TR was positive in 36 of 42 (85.7%) patients with a PSA < 4.0 ng/ml. In contrast, PSA was positive in 12 of 29 (41.4%) patients with a TR < 8.2 U/ml. In the assessment of validation cohort, serum TR also had greater AUC, sensitivity, and specificity values than did PSA and DRE in patients with PC compared with BPH (Table 2). Combined model was better than those alone. Based on the ROC curve, the serum TR levels (> 8.2 U/ml) as an indicator for auxiliary diagnosis of PC from BPH yielded a sensitivity of 82.8% and a specificity of 70.4%, with the area under the curve at 0.868 (95% CI, 0.819-0.905). Similarly, when PC patients were compared with BPH and normal cases, the AUC for TR was also larger than that for PSA (P < 0.001; Table 2).

In our study, 118 patients (53.6%) with BPH had a PSA level of 4-10 ng/ml and/or a suspicious DRE. However, only 14 (6.4%) patients with BPH had a serum levels of TR > 8.2 U/ml. Thus, 104 (47.2%) of the patients with BPH did not need biopsy.

The relation between TR and metastasis, tumors grade

In the test and validation cohort, 78 PC patients were with metastasis. The median level of serum TR in those patients was 25.2 (17.2-34.0) U/ml, which was significantly higher than that of patients without metastasis [13.0 (8.0-20.3) U/ml; P < 0.0001; Figure 3A]. There was no different of PSA in those two groups (P = 0.321). Based on the ROC curve, the optimal cutoff value of serum TR levels as an indicator for auxiliary diagnosis of metastasis was projected to be 18.0 U/ml, which yielded a sensitivity of 66.9% and a specificity of 80.4%, with the area under the curve at 0.746 (95% CI, 0.682-0.823; Figure 4A). In regression analysis, we calculated the OR of TR levels as compared with tumors grade, age, family history,
TR level as potential biomarker for PC

history of smoking, BMI, DRE findings, AFP and Hs-CRP. With an unadjusted OR of 11.55 (95% CI, 4.43-26.15), TR (> 18.0 U/ml) had a strong association with metastasis. After adjusting for all other significant outcome predictors, TR remained an independent metastasis predictor with an adjusted OR of 4.99 (95% CI, 2.64-8.09). In addition, age, DRE and Hs-CRP remained significant metastasis predictors.

In our study, 109 patients (38.9%) were classified as HGT. The median level of serum TR in those patients was 25.0 (15.5-37.2) U/ml, which was significantly higher than in LGT [14.2 (8.0-21.0) U/ml; P < 0.0001; Figure 3B]. Similarly, the median level of serum PSA in HGT was significantly higher than in LGT [P < 0.0001]. Based on the ROC curve, the optimal cutoff value of serum TR levels as an indicator for auxiliary diagnosis of HGT was projected to be 17.5 U/ml, which yielded a sensitivity of 73.5% and a specificity of 66.8%, with the area under the curve at 0.758 (95% CI: 0.694-0.829; Figure 4B). Again, in regression analysis, we calculated the OR of TR levels as compared with metastasis, age, family history, history of smoking, BMI, DRE findings, AFP and Hs-CRP. With an unadjusted OR of 12.68 (95% CI, 4.15-28.19), TR (> 17.5 U/ml) had a strong association with HGT. After adjusting for all other significant outcome predictors, TR remained an independent HGT predictor with an adjusted OR of 5.15 (95% CI, 2.52-9.14). In addition, age, metastasis, DRE and Hs-CRP remained significant HGT predictors.

Discussion

There are several lines of evidence suggesting that OS is linked to the development of PC [16, 29]. PC is commonly associated with a shift in the antioxidant-prooxidant balance towards increased OS. Previous studies highlighted the altered prooxidant-antioxidant status in prostatic tissue of man, rat and also in cell lines, where the imbalance between these antagonist played a major role in the initiation of prostate carcinogenesis [30]. Another major component involved in the maintenance of redox balance in the cell is the glutathione oxidation-reduction system. Somatic mutations, causing inactivation of the glutathione S-transferase gene (GSTP1) have been identified in almost all the prostate cancer cases examined by Nelson and colleagues [31]. Therefore, the sensitive balance between the oxidant and antioxidant components of the cells and their regulatory mechanisms seem to play a major role in developing a malignant state in prostate tissue. In the present study, we first evaluated the different serum levels of TR, an OS biomarker in Chinese PC and BPH patients.

In our study, we firstly suggested that serum TR may be a novel marker of PC in Chinese sample. The ROC curve comparing patients with BPH showed that TR was superior to PSA in diagnosing PC with an AUROC of 0.86 (95% CI: 0.82-0.9700) and a sensitivity of 81.8%, and a specificity of 68.9% (PSA: 0.63). Importantly, 85.7% of the PC patients with PSA < 4.0 ng/ml had a positive TR. In addition, the level of TR tended to increase as PC disease progressed as defined by metastasis and tumor grade. Similarly, previous studies had suggested that OS markers play importantly role in the PC diagnose, prevention and treatment. Antognelli et al. [32] pointed out a significant role for Glyoxalase 1 in PC progression, providing an additional candidate for risk assessment in PC patients and an independent prognostic factor for survival. Another study found that higher levels of plasma carboxymethyllysine (CML) were associated with increased risk of prostate cancer [33]. Barocas et al. [34] reported that F2-isoprostanes may also serve to estimate the efficacy of interventions targeting OS mechanisms in PC prevention or treatment. Kumar et al. [35] suggested that therapies aimed at reducing ROS production might offer effective means of combating prostate cancer in particular.

PSA is one of the most widely used biomarkers for PC. So far, it seems unlikely that PSA can be replaced as first line screening parameter within the next years. Although PSA is an organ-specific marker, it is not disease-specific and raised serum levels can occur in many benign conditions such as BPH or prostatitis as well as manipulations (bicycling, digital rectal examination, and catheterization) of the prostate can also cause elevated PSA serum concentrations [36]. Currently, early detection of PC relies primarily on an abnormal DRE and an elevated PSA level leading to a prostate biopsy [37]. However, because of the low specificity of PSA, up to 75% of men with PSA levels of 4-10 ng/ml
and/or a suspicious DRE have a negative first biopsy [38]. Thus, the vast majority of patients subjected to TRUSPB have negative results, so there is clearly a need for better means to select whom to biopsy. There have been several modifications to serum PSA assessment to improve the test’s performance, including recourse to age-specific PSA levels, PSA density, and free-to-total PSA ratios [39]. Meanwhile, many biomarkers have been developed, such as: free PSA (fPSA) and A [2] pro PSA [40, 41], the fusion gene (TMPRSS2-ERG), sarcosine, GSTP-1 hypermethylation, and PCA3 [37, 42]. In our study, according to the results of TR, 104 (47.2%) of the patients with BPH did not need unnecessary biopsies. This will significantly reduce the rate of over-detection and treatment.

Whether higher circulating level of TR is an accelerator or only is a marker of PC remains uncertain. It is important to discuss whether TR in PC patients has pathological roles or just is as indicator of OS or inflammation. OS is implicated in prostate cancer by several lines of evidence. First, an increase in the generation of ROS from the metabolism of endogenous and exogenous compounds within prostatic cells can produce DNA adducts or directly damage DNA [43]. If these alterations in the DNA lead to the activation of oncogenesis or the inactivation of tumor suppressor genes, cancer may develop. Second, although ROS play a role in the initiation of prostate cancer either by directly affecting nuclear DNA, they also create mutations in mitochondrial DNA (mtDNA), which in turn are believed to promote aging [44]. With age, an accumulation of somatic mutations in mtDNA causes deficiencies in oxidative phosphorylation and the electron transport chain, which in turn cause both increased production of ROS and their leakage into the cytoplasm. The accumulation of mtDNA somatic mutations seems to be an indicator of human age related disorders, including prostate cancer [16]. Third, prostatic cells must maintain a redox balance between the generation of ROS and oxidative defenses that quench free radicals or conjugate them to glutathione [45, 46]. Either an increase in ROS production or a decrease in antioxidant capacity may disrupt this balance, promoting prostate carcinogenesis [46, 47]. Fourth, exciting information points to an essential role for increased ROS generation in several cellular processes associated with neoplastic transformation and aberrant growth and proliferation [48, 49]. Processes associated with proliferation, apoptosis, and senescence may be a result of the activation of signaling pathways in response to intracellular changes in ROS levels [50]. Thus, excessive production of ROS or inadequacy in a normal cell’s antioxidant defense system (or both) can cause the cell to experience oxidative stress and the increased ROS may play a broader role in cellular processes associated with initiation and development of many cancers including prostate cancer. Lastly, TR may play role through inflammation. The strongest evidence for an inflammatory component in prostate carcinogenesis is based on the characteristics of a precursor lesion, proliferative inflammatory atrophy, which is an area of highly proliferative but atrophic epithelial cells with notable inflammatory infiltrates [51]. Thus, TR might play important role in the process of PC rather than just was a diagnosis marker.

There are several limitations in this study. First, sample size is one of the most concerning issues. In our study, small sample and single center were included. Larger sample size is warranted to confirm the conclusion. Second, we cannot assess the prognosis value of TR because follow-up information of these patients was not yet available. Third, without serial measurement of the circulating TR levels, this study yielded no data regarding when and how the change of serum levels in these patients. In addition, TR measurements were performed after the PC and may not accurately reflect pre-PC exposure. Fourth, we measured only TR, which is a single antioxidant defense parameter. Effective antioxidant protection is provided by the cooperative and sequential actions of several antioxidant enzymes, and non-enzyme antioxidant molecules. So we could not determine the association of those factors with TR levels and PC. Fifth, in the present study, the classical determination of TR by ELISA method is widely used, which is considered ‘gold standard’. However, it is widely known that human serum contains immunoglobulins that affect the results of immunoassays by binding to reagent antibodies used in the assay. Lastly, we measured TR in serum, not in histologic specimen. It is still uncertain whether peripheral TR levels reflect similar changes in the liver
tissue. However, we did not get relevant information in this study. The relationship between peripheral and tissue TR levels warrants further investigation.

**Conclusion**

In conclusion, to the best of our knowledge, this is the first study to report the clinically diagnostic relevance of TR as a serum protein marker for PC in Chinese sample. Nevertheless, our study has demonstrated the additional benefit of TR measurement in the diagnosis of PC in the Chinese population. Further studies of the application of TR in this region may be beneficial. Moreover, TR levels were related with disease progressed in this subset of patients, suggesting that this marker may be a further tool not only for diagnosing PC but also for predicting.

**Acknowledgements**

We also express our gratitude to all the patients who participated in this study, and thereby made this work possible. Authors also acknowledge the contribution of editors and the reviewers who have helped us to improve the manuscript.

**Disclosure of conflict of interest**

None.

**Abbreviations**

PC, Prostate cancer; PSA, Prostate-specific antigen; DRE, Digital rectal examination; BPH, Benign prostatic hyperplasia; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; OS, Oxidative stress; TR, Thioredoxin reductase; BMI, Body mass index; Hs-CRP, High-sensitivity C-reactive protein; HGT, High-grade tumors; LGT, Low-grade tumors; IQR, Interquartile ranges; OR, Odds ratios; CI, Confidence interval; ROC, Receiver operating characteristic; AUC, Area under the curve.

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